STRUCTURE OF HIV-1 REV BINDING ELEMENT (RBE)

Novel Base Pairing and Unexpected Flexibility in Key HIV Genetic Domain

One of the most promising strategies for treating infection by human immunodeficiency virus type 1 (HIV-1) is to interfere with the replication of the virus at the genetic level. To this end, researchers from Berkeley Lab's Physical Biosciences Division, using the Macromolecular Crystallography Facility (a component of the Berkeley Center for Structural Biology), have analyzed the structure of a portion of the HIV-1 gene (the Rev binding element, or RBE) that plays a key role in the virus's life cycle. The high-resolution (2.1-angstrom) structure obtained at the ALS facility indicates a greater degree of flexibility in the RBE than previously thought. Such details about the molecular structure of HIV-1 will help facilitate the design of drugs capable of throwing a metaphorical wrench into the virus's genetic machinery.

A virus reproduces itself by co-opting its host cell's own replicating mechanisms. Essentially, the virus enters the cell nucleus and splices its own DNA into that of the host. The modified host DNA now contains instructions for producing, not only the proteins needed by the cell, but the proteins required to assemble a new virus particle as well. In the case of HIV-1, one of these viral proteins, the Rev protein, must fit (bind) like a key into the RBE "lock" before viral replication can proceed. If this binding can be blocked, perhaps by a different molecular key of the right shape, the infected cell will no longer be able to produce new virus particles, thus slowing the spread of the infection.

The RBE is a highly structured region in the virus's messenger RNA, which is a mobile copy of the genetic information stored in DNA. Previous studies have shown that the RBE takes the form of a double helix that includes two "noncanonical" base pairs of nucleotides: guanine-adenine (G-A) and guanine-guanine (G-G). Normally, guanine will pair only with cytosine (G-C). The two noncanonical base pairs are separated by a single (unpaired) uracil (U). This arrangement distorts the double helix and presents a unique, high-affinity binding site for the Rev protein.

To study the structure of the RBE crystallographically, the researchers used synthetic RBE molecules. They heated solutions containing RNA strands that were chemically synthesized to have the requisite nucleotides in the proper order. The solutions were then purified, concentrated, and crystallized. A brominated derivative (where 5-bromouridine replaced one of the uracils) was also produced and used for crystallographic phasing. Analysis of the data, obtained using multiwavelength anomalous diffraction (MAD) techniques at Beamline 5.0.2, revealed four unique RBE structures that could be grouped into two types (I and II) distinguishable by structural deviations of up to several angstroms. The largest deviations (and thus the greatest flexibility) occurred at the "internal loop" where the noncanonical base pairs create an asymmetrical bulge in the double helix.

The four structural variants were found to differ in several respects from models previously derived using nuclear magnetic resonance (NMR) techniques. For example, in the type II crystal structures, the bonding between the noncanonical G-A base pair was unexpectedly bridged by water molecules. Furthermore, in both the type I and type II structures, the noncanonical G-G pair formed a novel asymmetrical bond, in contrast with the symmetrical bond indicated by NMR-based models of the RBE in complex with (i.e., bound to) the Rev protein. This means that the G-G bonds would have to be broken and re-formed to accommodate Rev protein binding. The observed flexibility of the bulged internal loop region may facilitate this kind of extensive structural change. These results suggest that the NMR model provides only a partial picture of how Rev binding occurs. More detailed studies will be needed before we can begin identifying or designing potential drugs to exploit the RBE-Rev interaction. ■

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L.W. Hung, E.L. Holbrook, S.R. Holbrook, "The crystal structure of the Rev binding element of HIV-1 reveals novel base pairing and conformational variability," *Proc. Natl. Acad. Sci. USA* **97**(10), 5107 (2000).

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- **Drug design motivation**
  - *RBE: RNA double helix with two noncanonical base pairs (G-A, G-G)*
  - *Rev protein must bind to RBE before virus can reproduce*
  - *Finding a molecule to bind in place of Rev would prevent replication*
- **RBE structure at MCF Beamline 5.0.2**
  - *Synthetic RBE purified, concentrated, crystallized*
  - *Brominated derivative used for phasing*
  - *Structure determined to 2.1 Å using MAD techniques*
- **Unexpected structural details**
  - *Four unique structural variants, with two basic types (I and II)*
  - *Novel base pairing, high flexibility*
  - *Rev binding possibly requires breaking of bonds and reorientation of molecules*

L.W. Hung, E.L. Holbrook, S.R. Holbrook (Berkeley Lab). [*Proc. Natl. Acad. Sci. USA* **97**(10), 5107 (2000)]

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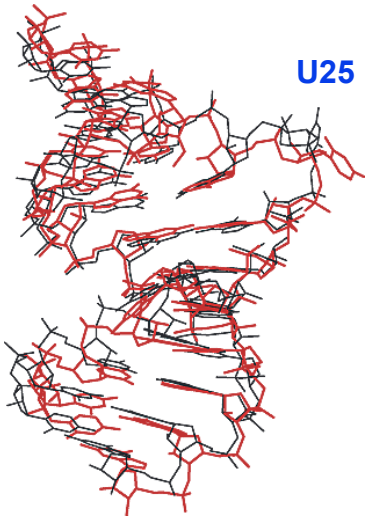
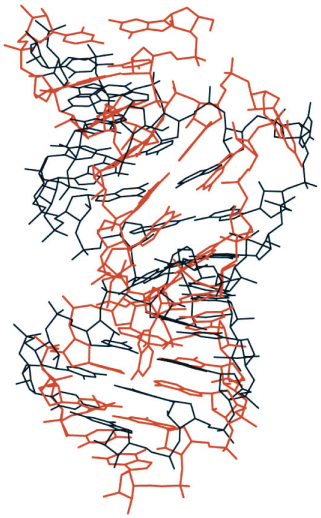
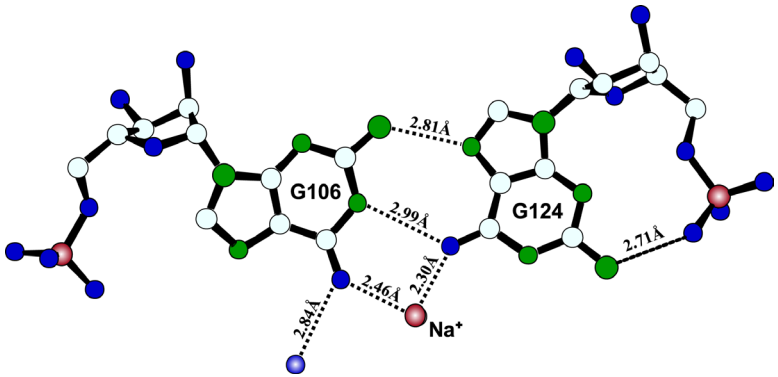
Superposition of type I (black) and type II (red) RBE structures.





Superposition of type I RBE structure (black) and NMR model of unbound RBE (orange).

The unpaired uracil (U25) is located between two noncanonical base pairs in an asymmetrical bulge in the double-helix structure.



Asymmetrical bonding between noncanonical G-G base pair.

L.W. Hung, E.L. Holbrook, S.R. Holbrook (Berkeley Lab). [*Proc. Natl. Acad. Sci. USA* **97**(10), 5107 (2000)]